Less Lipoproteins, Higher Purity: Why You Should Consider Platelet EVs over Plasma EVs

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Introduction

Blood plasma EVs studies are hindered significantly by lipoproteins co-existing with them, forming >99% of blood plasma constituents [2,3]. With similar characteristics to EVs, the abundance of lipoproteins challenges the labeling and detection blood plasma EVs [4]. Contrarily, since the vast majority of plasma EV population is released from platelets, platelet-derived EVs form a superior source for theranostic approaches without contamination issues from lipoprotein populations.

Combining nanoparticle tracking analysis (NTA) and dual angle light scattering, DAISY (Holtra AB) reports not only size and concentration but also single particle refractive index [5]. This allows fast and straightforward differentiation of particle types within a mixture. This makes DAISY a powerful tool to better understand complex nanoparticles, such as blood-derived EVs [6].

In this study, we demonstrate how HansaBioMed platelet EV standards show superior purity to their plasma-derived counterparts and how DAISY overcomes a significant bottleneck in blood EV analysis.

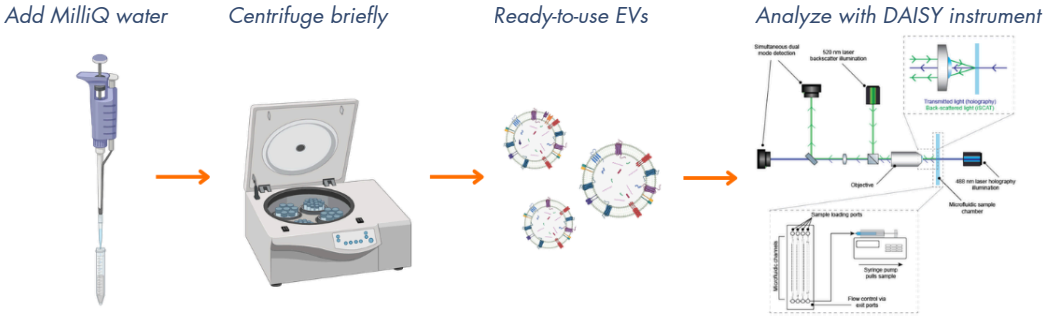

Figure 1: Study workflow including reconstitution of lyophilized EVs by HansaBioMed and analysis with DAISY instrument by HOLTRA

Materials and Methods

All EV samples were provided by HansaBioMed Life Sciences in lyophilized form. HEK293-EVs (Cell Derived EVs, SKU: HBM-HEK293-100/2) were purified by tangential flow filtration (TFF) in followed by size exclusion chromatography (SEC), whereas plasma EVs are purified only with SEC. Platelet derived EVs (SKU: HBM-PET-100/2) were purified using ion exchange chromatography (IEX) and TFF.

EVs were re-suspended in 0.1µm filtered PBS according to HansaBioMed's instructions. All EV samples were subsequently diluted to an approximate concentration of 1 x 10^9 particles/ml for DAISY analysis. VLDL (LP1, Sigma-Aldrich) was diluted to 0.5 µg/ml, to a concentration of ~10^9 particles/ml.

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For analysis with DAISY instrument (Holtra AB), 10 µl of dilute sample was injected into the microfluidic chip. A constant flow rate of ~90 µm/second was established and 50 seconds of video was acquired for each sample. Data were acquired and analyzed with HoloViz software (Holtra AB).

Results

The majority of existing analytical methods for determining EV size and concentration are unable to distinguish between EVs and co-purifying contaminants such as lipoproteins. This is due to the overlapping size distribution of small EVs and VLDL (Fig 2.A). Despite their overlapping size distribution, DAISY analysis is able to segregate EVs and VLDL based on the relationship between particle size and refractive index (Fig2.B).

By classifying and quantifying particles based on their size and refractive index, DAISY is able to accurately determine the proportion of EV in a sample (Fig.3). A standard curve was constructed by carrying out DAISY analysis of cell derived EV and VLDL mixed in known proportions (Fig.3A&B). The relationship between the proportion of particles classified as EV ("Proportion EV gate") and the known proportion of EV in the sample was highly linear across the full range of EV proportions (0 to 100% EV nominal, R^2 0.99) (Fig. 3.B). DAISY analysis of plasma-derived and platelet-derived EV showed a marked difference in their respective refractive index distributions (Fig.3C). Applying EV classification and quantitation revealed that whilst the plasma-derived EV sample consisted of approximately 28% true EV particles by number, approximately 96% of the particles in the platelet-derived EV sample were classified as EV (Fig.3D).

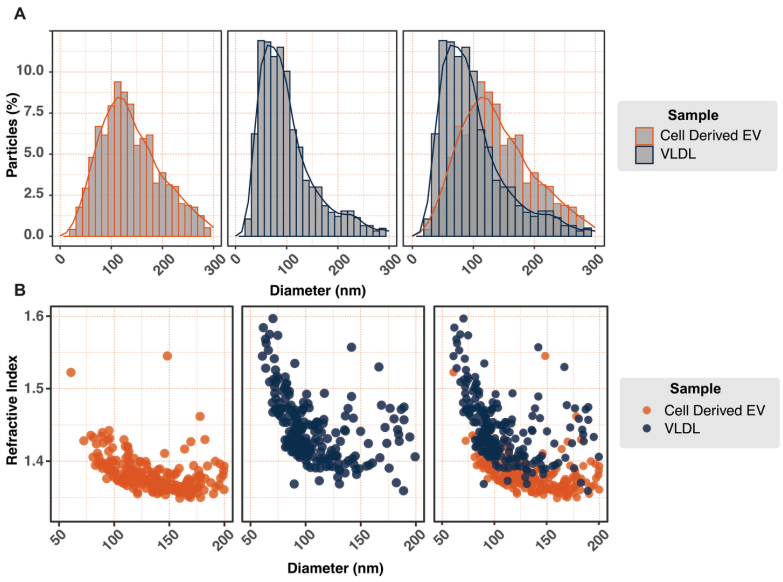

Figure 2: DAISY differentiates EV from VLDL based on size & refractive index. (A) Number-based size distributions for cell derived EV and VLDL particle populations measured by DAISY with the probability density function overlaid. VLDL and cell derived EVs have an overlapping size distribution which precludes quantitation based on size alone. (B) Hydrodynamic size vs refractive index for cell-derived EV and VLDL particle populations measured by DAISY. By measuring the refractive indices of individual particles as well as their hydrodynamic size, DAISY is able to segregate EVs from VLDL.

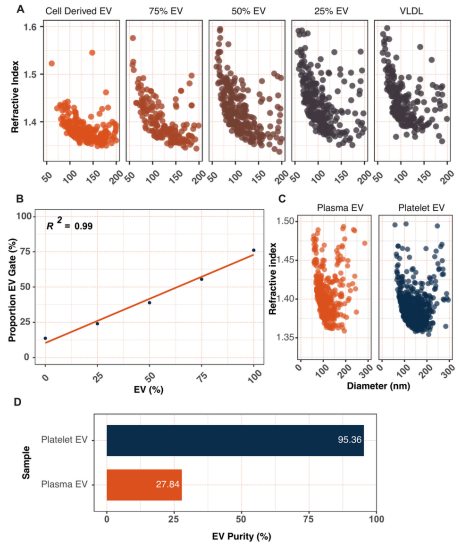

Figure 3: EV quantitation in heterogenous samples using DAISY. (A) Size and refractive index measurements of particle populations comprising cell-derived EV and VLDL mixed in the stated ratios. (B) Proportion of particles classified as EV, based on their size and refractive index, for each mixture. The relationship between EV classification and nominal EV proportion is highly linear (R^2 = 0.99). (C) Size and refractive index of particles from samples of plasma derived and platelet derived EV. (D) Result of EV classification and quantitation of platelet-derived and plasma-derived EV sample particles. Platelet-derived EV sample shows greatly increased proportion EV as compared to plasma-derived EV sample.

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Conclusion

By differentiating between EV and lipoprotein subfractions through refractive index information, DAISY analysis confirms that platelet derived EVs offer a high purity alternative to plasma-derived EVs. With such priority, platelet EVs form a suitable reference material for blood EV research.

References

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